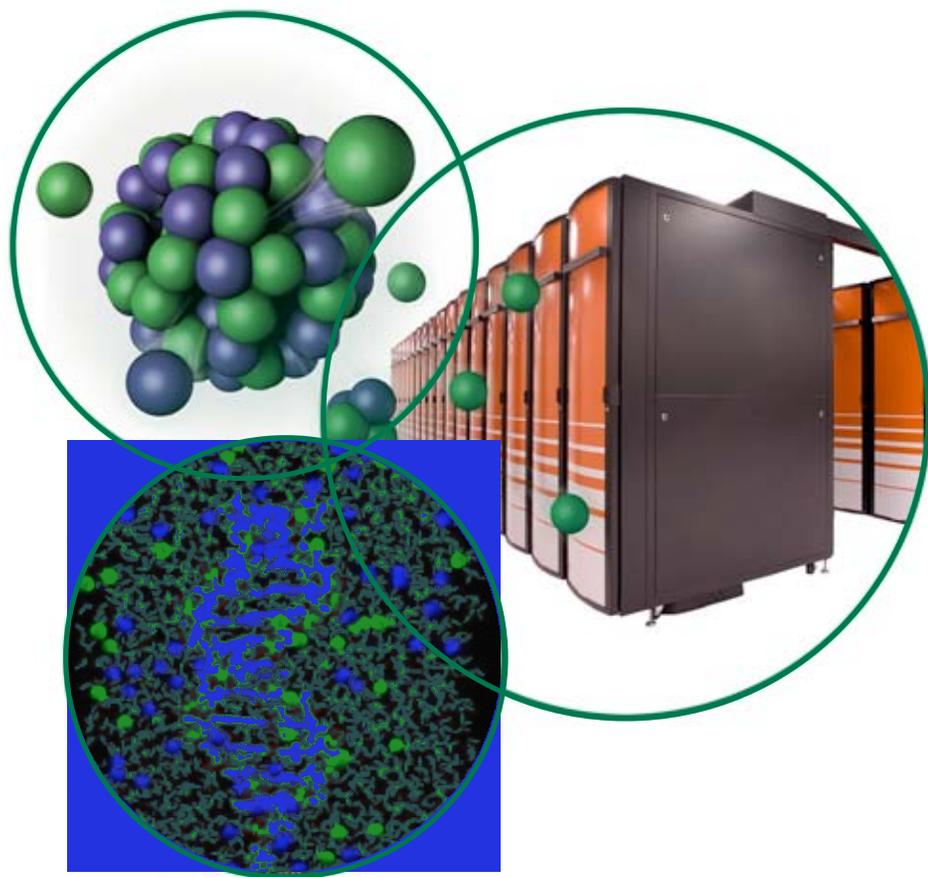


Geochemical and Molecular Mechanisms Controlling Contaminant Transformation in the Environment

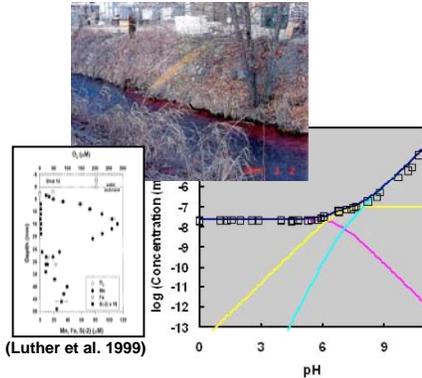


**ERSP 3rd Annual PI
Meeting, April 7 – 9, 2008**

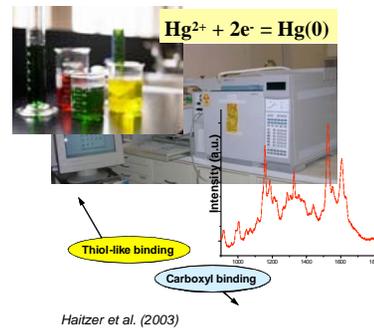
ORNL ERSP Science Focus Area (SFA)

Strategy for understanding contaminant transformation and environmental behavior

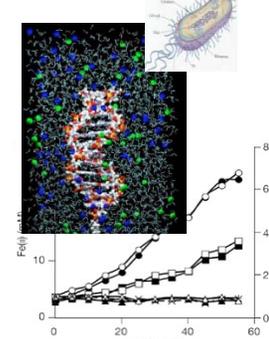
Field biogeochemistry



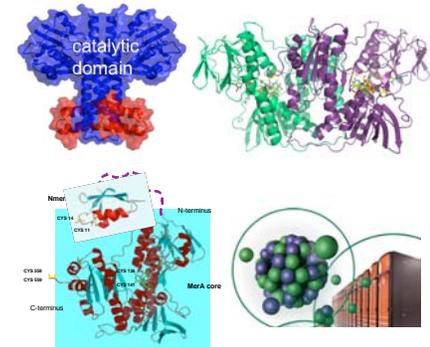
Fundamental rates and mechanisms



Microbial and genetic controls



Molecular structure and simulations



Transformation in field

Speciation & mechanisms

Molecular dynamics

Sediment-water interface
Species/ abundance
Microbial communities

Coupled microbial
and geochemical
reactions

Molecular level understanding
of contaminant association
and reaction

Reaction mechanisms and kinetics at groundwater–surface water interface

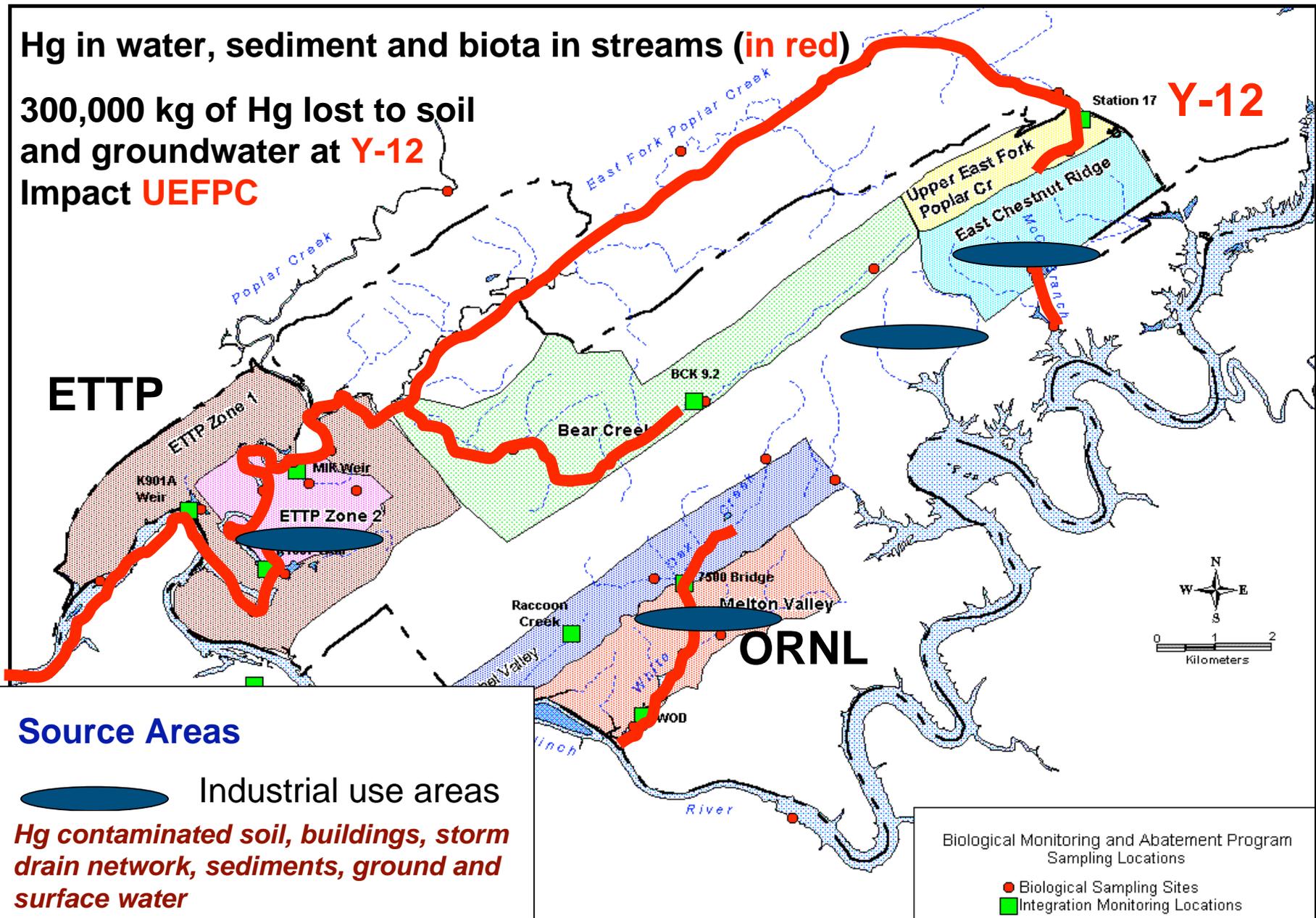
Research focus and goals

- Mercury – the net balance of methylation and demethylation
 - **Geochemical/biological controls on Hg speciation and transformation, and how and what Hg precursors are produced, transported and methylated**
 - **Enzymatic mechanisms of transformation between major Hg species and methyl mercury.**
- Uranium – stability in subsurface
 - **Microbial oxidizers – Rates and mechanisms in the oxidation of U(IV) minerals**
 - **Structure and function of key heme proteins required for direct electron transfer, microbial-mineral interface models**

Mercury concerns at Oak Ridge Reservation

Hg in water, sediment and biota in streams (in red)

300,000 kg of Hg lost to soil and groundwater at Y-12
Impact UEFCPC



High mercury concentrations in biota

- **High concentrations of elemental and Hg(II) complexes in shallow soils near industrial infrastructure**
- **Oak Ridge environment: strong groundwater/surface water interactions (>50" annual rainfall)**
- **Methyl mercury is readily accumulated and can increase up the food chain**
- **Hg exceeds regulatory limits—new standards could significantly impact Y-12 operations and costs**
 - TN TDEC developing an East Fork Poplar Creek TMDL; focus on loading/flux, not concentration
 - TN recently lowered Hg level that triggers an advisory
 - EPA concern for ecological risks
- **Modernization of facilities could result in increased transport of Hg to streams**



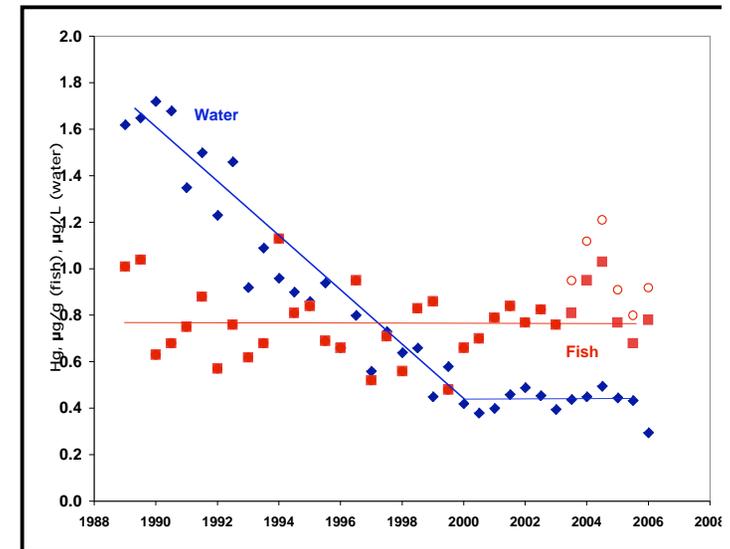
EFPC downstream of Y-12

Source control has not lowered Hg in fish

- Mercury bioaccumulation in fish is not proportional to concentrations of waterborne Hg

Examples of water/ fish disconnect (see poster):

Oak Ridge Sites	Hg in water (ng/L)	Hg in fish (mg/kg)
White Oak Creek	60	0.5
EF Poplar Cr (upper)	400	0.8
EF Poplar Cr (lower)	100	0.8
Bear Cr	1–3	0.7
Rogers Quarry	1	1.1
Reference site	1–3	0.2



- Hg in fish correlates with methyl Hg in water, but not with total Hg in water. So, at contaminated sites, there is no model relating methyl Hg and total Hg in water

- Not possible to eliminate inorganic Hg inputs; alternative strategies to reduce methylation may be only means to reach fish concentration targets

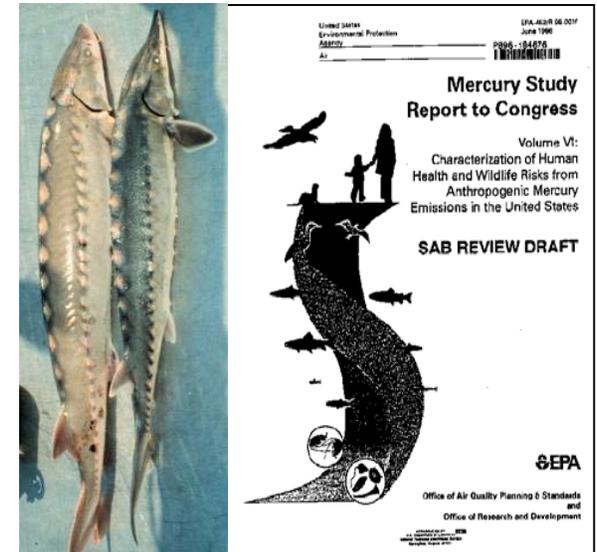
Basic research needs: elucidate Hg methylation processes at sediment-water interface and the controls on methyl Hg production



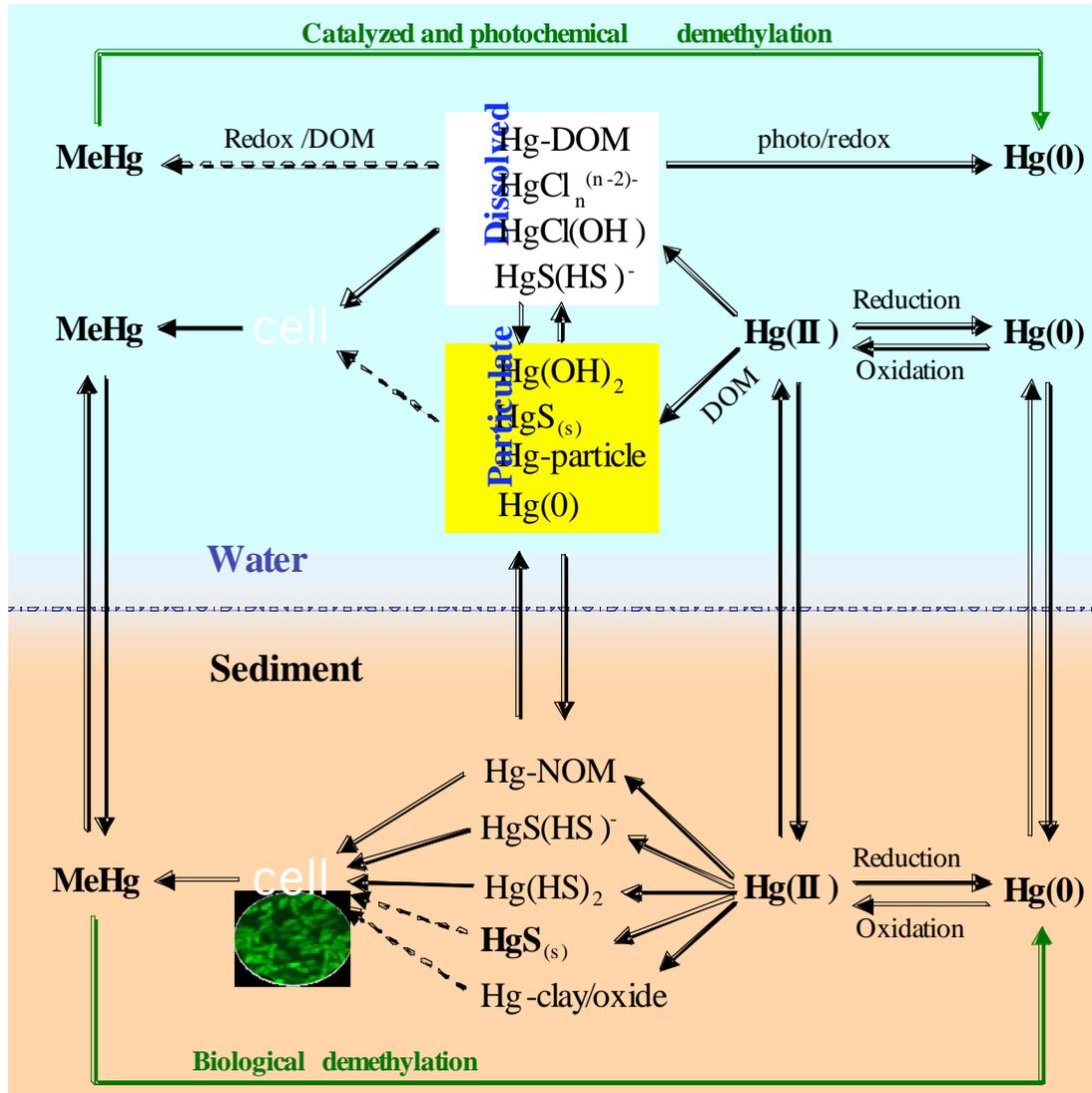
The mercury challenge

National:

- **Global pollutant readily transported and re-emitted**
- **Highly toxic to human and ecological receptors**
 - **Methylmercury (MeHg) is a potent human neurotoxin, highly bioaccumulative**
- **Hg found at all DOE sites; waste and environmental issues at many (e.g., Savannah River, Paducah...)**
- **Complex chemistry /speciation /methylation–demethylation processes**
- **Hg at industrial contaminated sites**



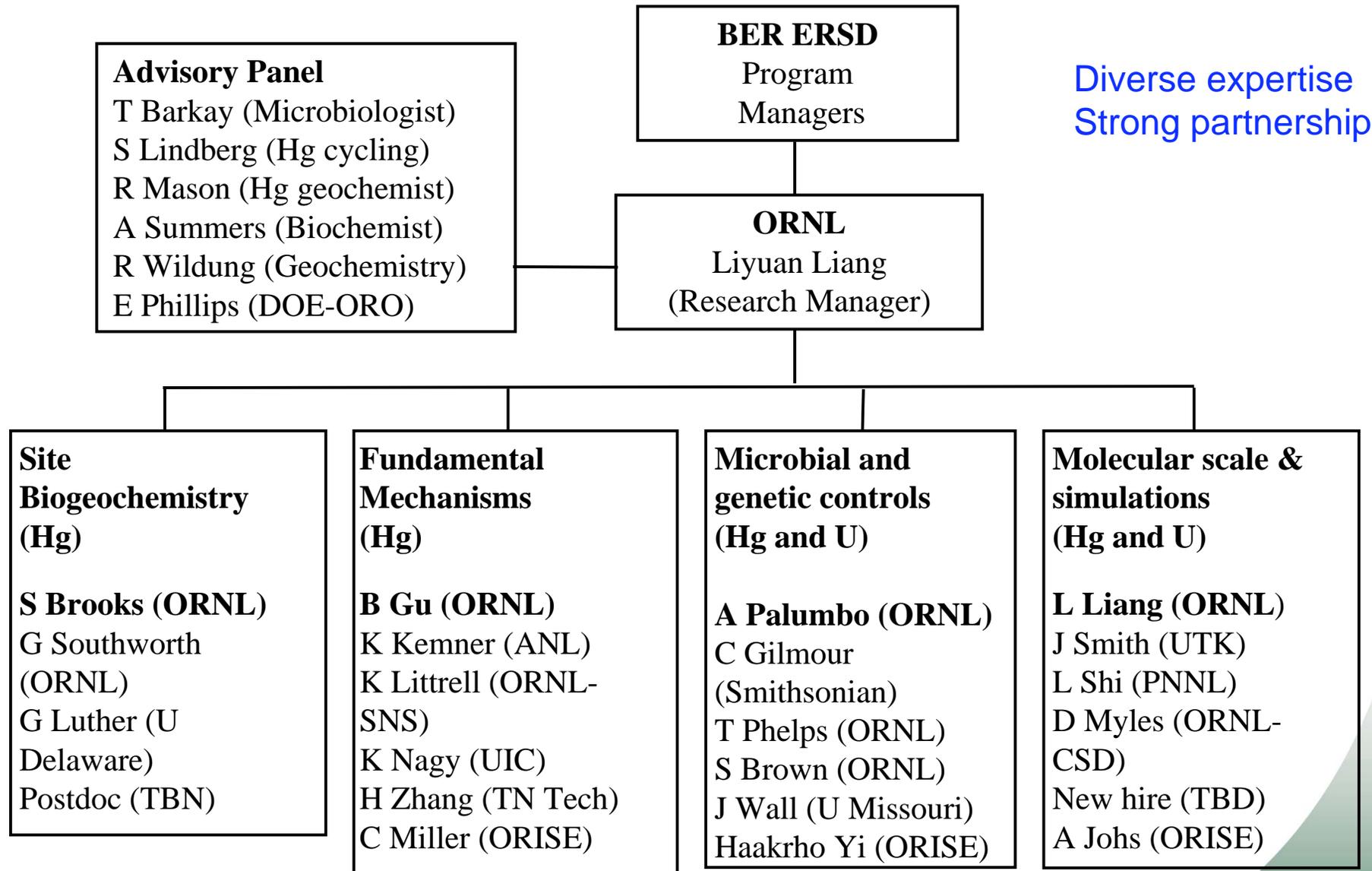
Hg methylation at sediment-water interface



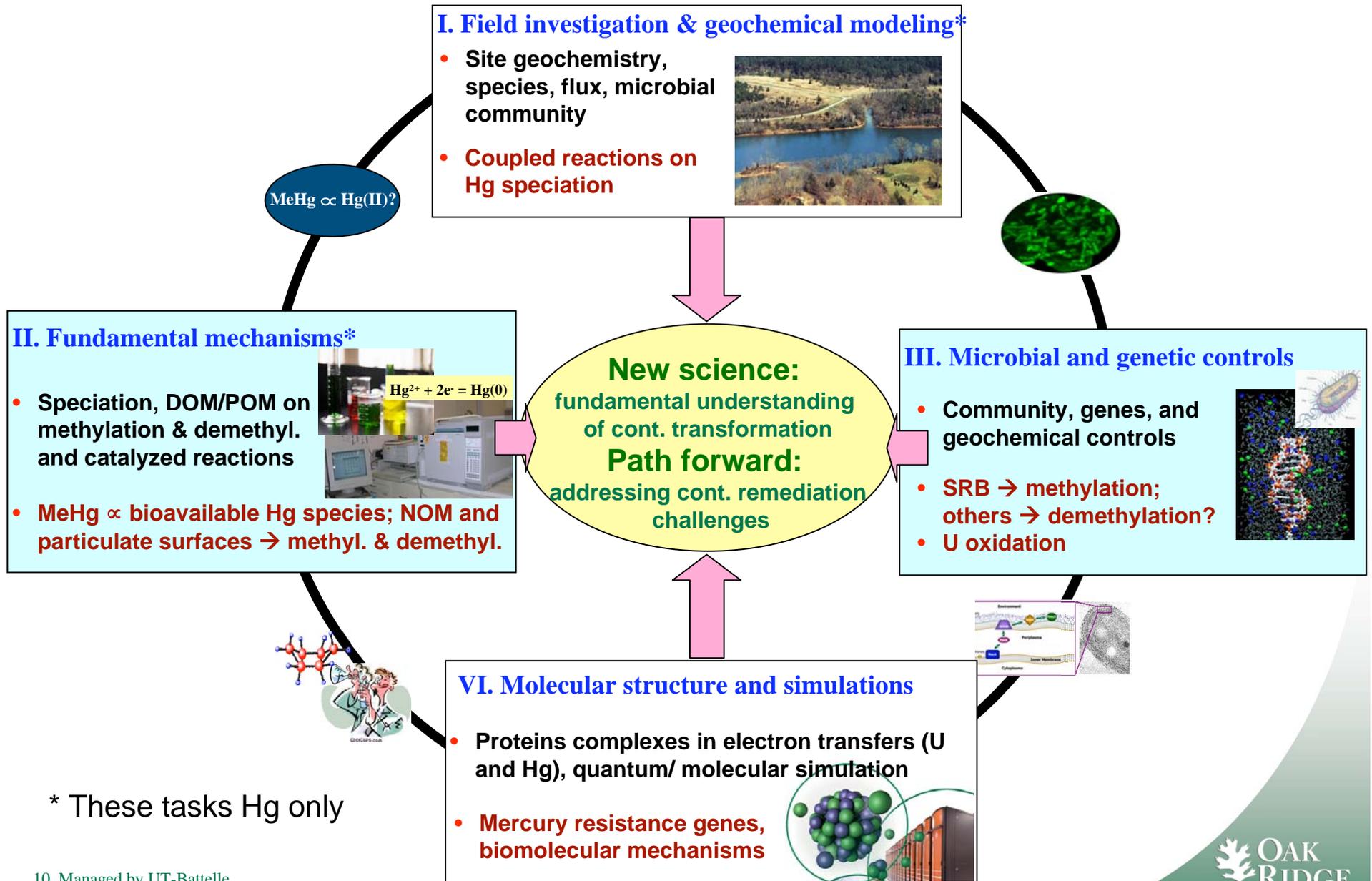
Need to elucidate:

- Oxidation, reduction, and species transformation
- Dominant chemical species and bioavailability
- Abiotic /biotic methylation and demethylation
- Biochemical pathways for methylation and demethylation
- Coupled biogeochemical reactions – sorption, complexation, precipitation, stabilization, fate and transport
- Surface catalyzed and photochemical reactions

Research team

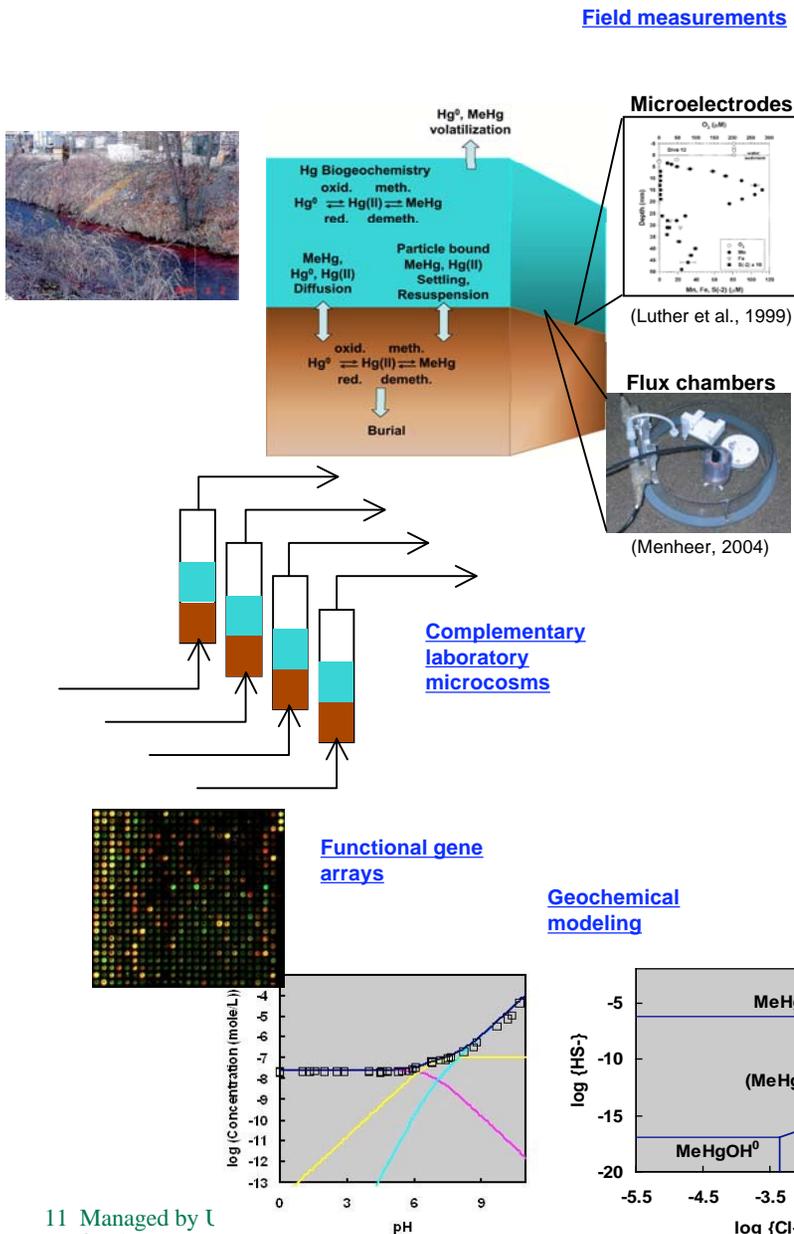


Integrated research approach



* These tasks Hg only

I. Site biogeochemistry



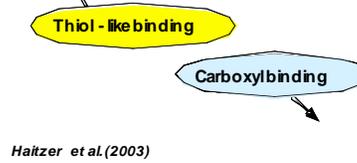
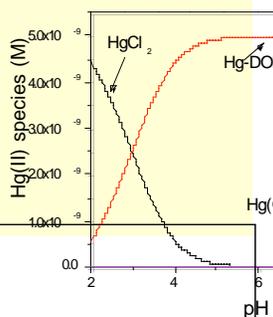
- Field studies (UEFPC)
 - Measurement of Hg flux
 - Quantify geochemical gradients
 - Benthic flux chambers
 - EXAFS and XANES analysis
- Microcosm Studies
 - Stable Hg isotopes to facilitate analysis
 - Transformation pathways
- Microbial Community Structure at the sediment-water interface
 - Functional gene arrays
 - Principal microbial communities
- Geochemical Modeling



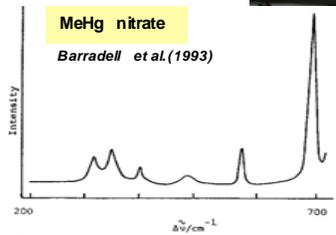
*** Critical understanding of Hg flux, biogeochemical controls, and microbial determinants**

II. Fundamental mechanisms and transformations

Hg(II) speciation: H^+ – Cl^- – CO_3^{2-} –DOM



Haitzer et al. (2003)



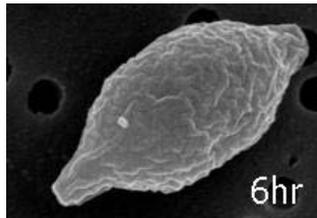
MeHg nitrate
Barradell et al. (1993)



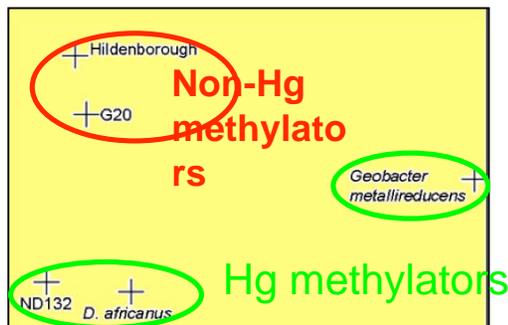
- Determine speciation and abiotic controls
 - Rates and mechanisms, oxidation/reduction
 - Single reactant to multi-component systems
 - Real-time spectroscopic analysis coupled with CVAA or CVAFS analysis
- Establish roles of DOM and POM in Hg methylation, demethylation, complexation, and stabilization of particulate Hg species
 - Specific moieties and functional groups
 - Labeled isotope studies; EXAFS and XANES analysis, speciation and coordination chemistry
 - Species, models, and effects on bioavailability
- Surface catalyzed and photochemical reactions
 - Roles in methyl. and demethylation
 - Sorbed species and reactions

*** Critical understanding of dominant Hg species, its bioavailability, and biogeochemical controls on rates and mechanisms of Hg methylation and demethylation**

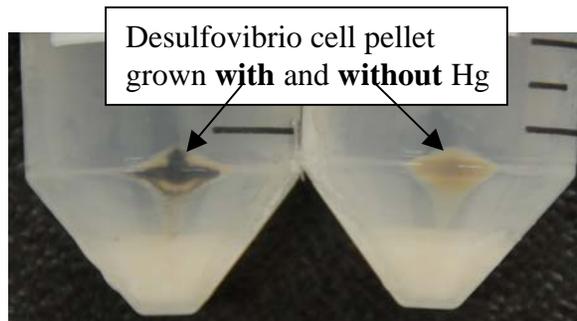
III. Microbial and genetic controls on mercury methylation



Desulfovibrio africanus
(SEM by Dwayne Elias, U of Missouri)



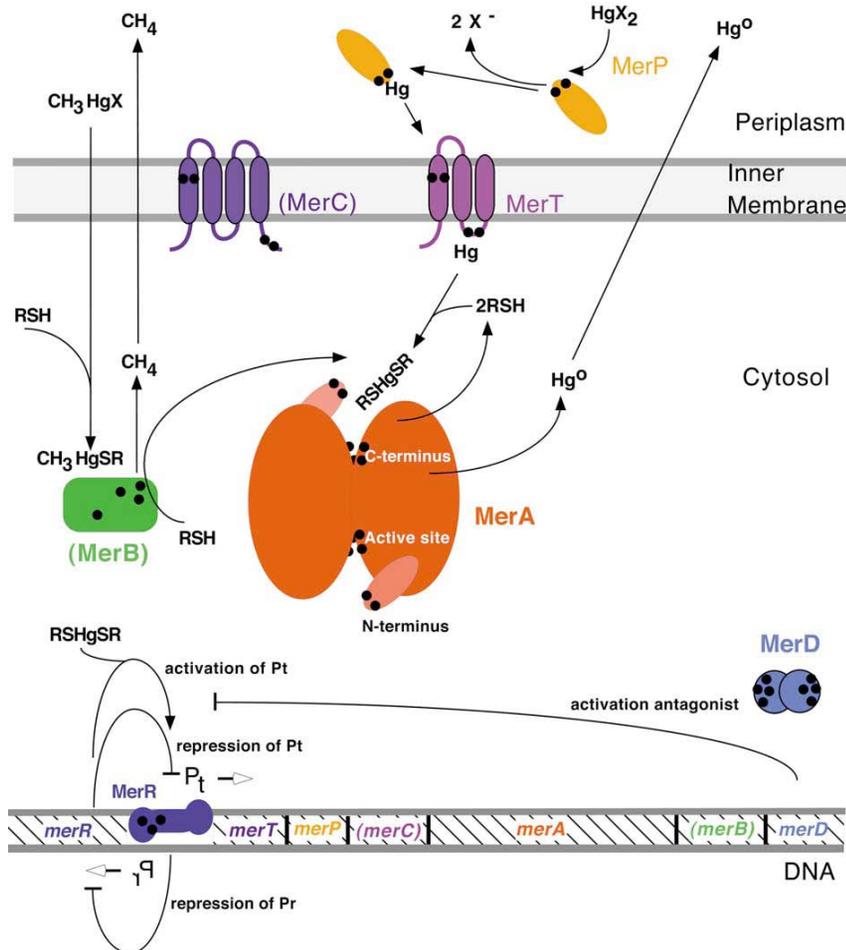
Genome wide PCA of COGs



- **Elucidate the genetic determinants of methyl Hg production and regulation**
 - Comparative gene expression, mutagenesis, and complementation
- **Determine the effect of geochemical factors on gene regulatory networks for mercury methylation**
 - Use whole genome microarrays to examine both biotic and abiotic effects on the methylating and nonmethylating *Desulfovibrio* transcriptomic profiles
- **Examine relationships among community structure, geochemical conditions, and methyl Hg production in sediments collected from Hg-contaminated sites**
 - Functional gene arrays
 - 16S rRNA gene clone library analysis

***Critical understanding of the genetic basis of the methylation and demethylation processes and the geochemical controls on microbial transformation.**

IV. Molecular structure and simulations

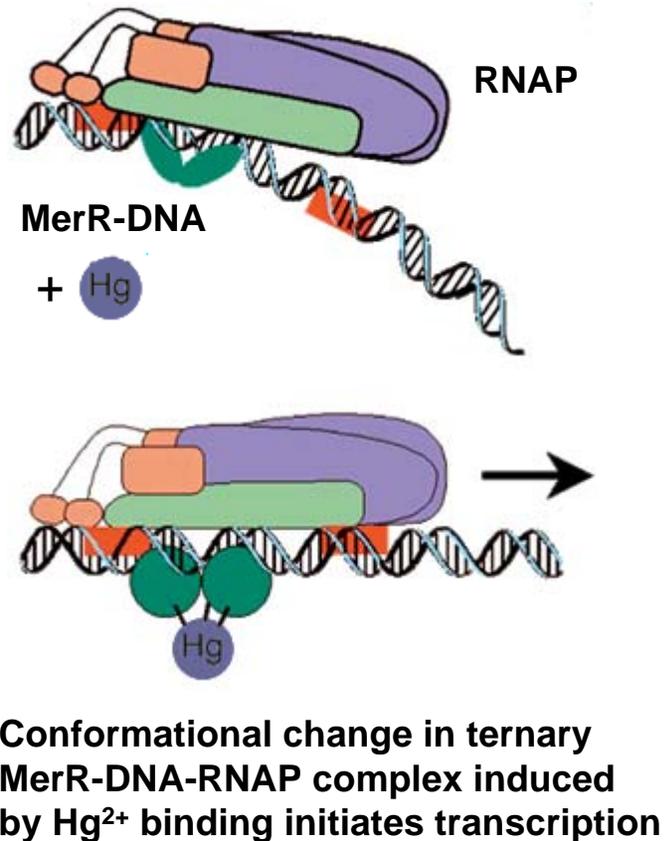


Barkay, T., S.M. Miller, and A.O. Summers: FEMS Microbiol Rev, 2003. 27(2-3): p. 355-84.

- Establish biochemical pathways in bacterial demethylation
- Obtain structure of protein/protein and protein/DNA complexes
 - **Apply small angle neutron scattering to reveal structure-function relationships**
- Reveal enzymatic mechanisms to understand the processes of demethylation and reduction
 - **Use quantum mechanical/molecular mechanical simulations**

*** Critical understanding of biomolecular mechanisms in Hg transformation (demethylation and methylation) by investigation of structure-function relationships**

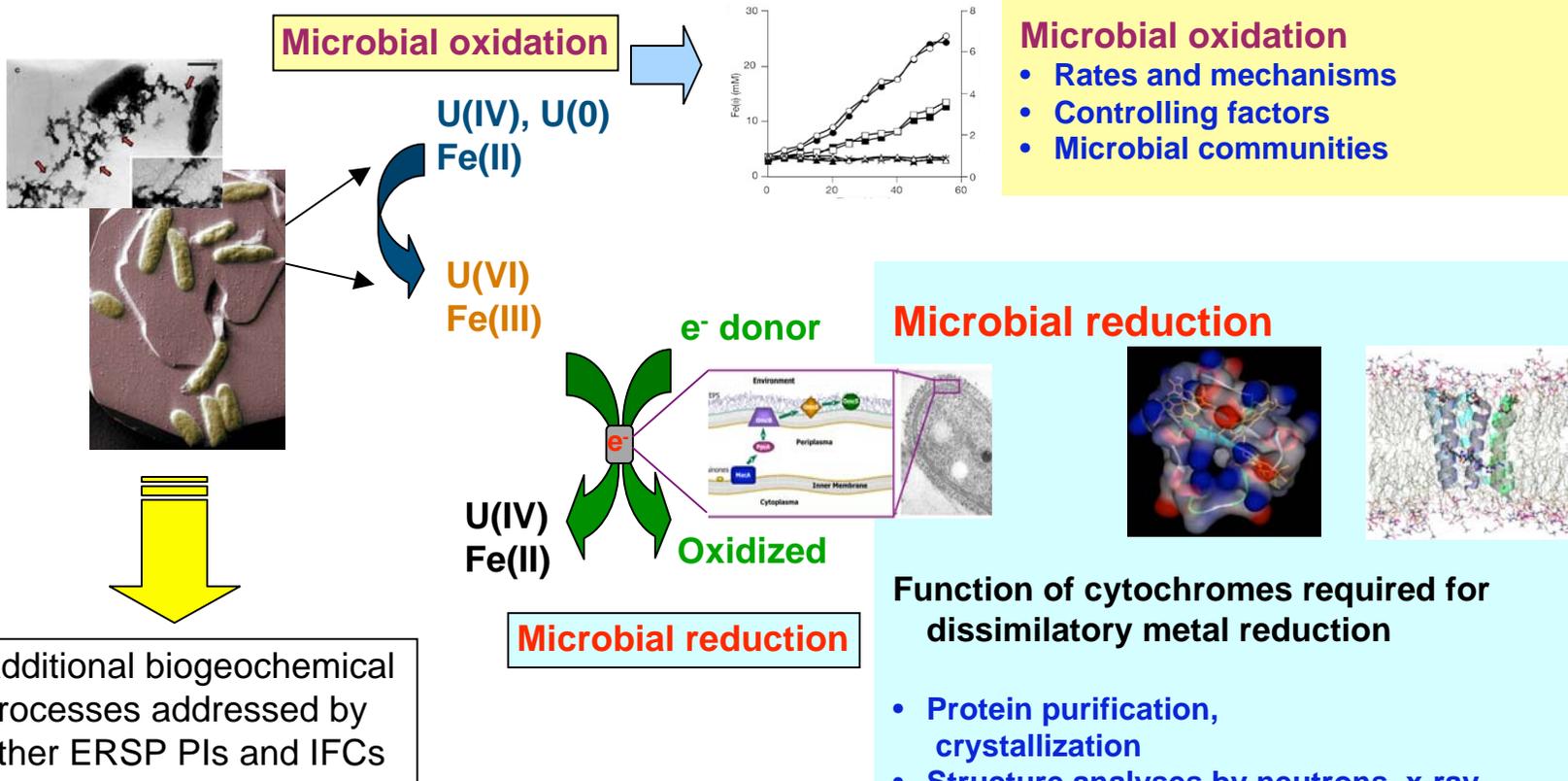
Small Angle Neutron Scattering to elucidate structure and function of molecular machines



- **SAXS/SANS for characterization of proteins and protein complexes**
- **Define protein shapes and compare solution and crystal structures**
- **SAXS/SANS is used to elucidate dynamic protein functional relationships**
- **SANS with contrast variation provides a method to reveal the orientation and location of specific components in complex biomolecular systems**

Figure adapted from: Brown, N. L., et al.: The MerR family of transcriptional regulators. FEMS microbiology reviews 27:145-163 (2003)

Uranium focus on microbial U oxidation and subcellular electron transfer processes



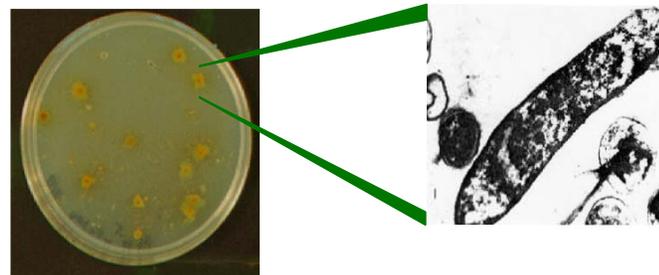
Zachara & Fredrickson, 2004; ERSP PI meeting

Reguera, G., et al.: Extracellular electron transfer via microbial nanowires. Nature 435:1098-1101(2005).

Role of metal oxidizing bacteria affecting U speciation

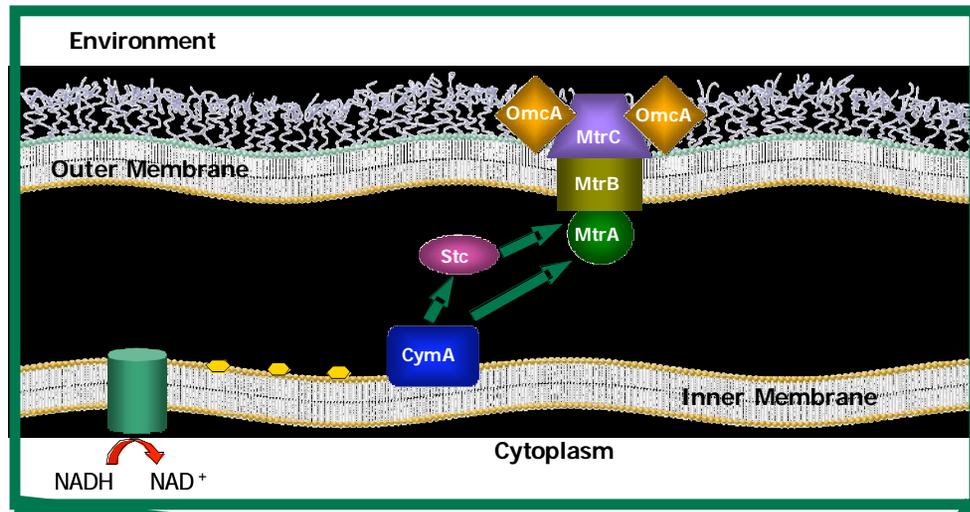
- Study microbial oxidation of U using site materials from Oak Ridge IFC
 - Use microcosms
- Use *Acidithiobacillus ferrooxidans* as a model organism to study genetic responses
 - Construct whole genome microarray
 - Conduct single and multi-factor experiments to investigate genetic responses to various geochemical conditions
- Ongoing work (see Phelps Poster)
 - Have obtained cultures of *Acidithiobacillus ferrooxidans* & testing pH tolerance in lab media
 - Designing the whole genome microarray and will print and test it this FY

***Critical understanding of role of specific metal oxidizing bacteria affecting U oxidation state and thus mobility in the subsurface**

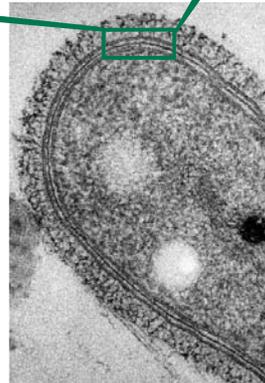
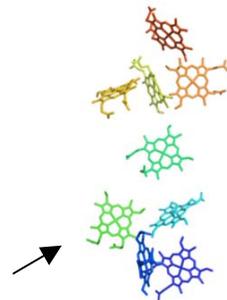
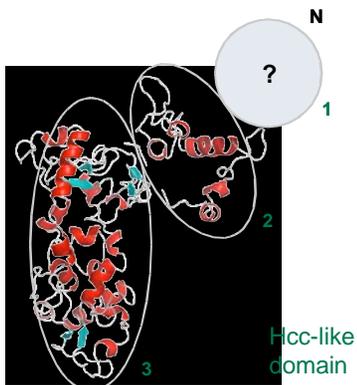


Acidothiobacillus ferrooxidans growing on culture plates. The cells themselves are colorless, the rust coloring associated with growing colonies results from the microbial production of Fe(III)

Cytochrome protein structure in dissimilatory metal reduction



- Elucidate structure of cytochrome complexes and structure function relationships
 - Use of small angle neutron scattering (SANS) to understand mechanisms of electron transport to minerals
- Investigate membrane insertion properties and interaction with mineral surfaces
 - development of a biomimetic bacterial-mineral interface model system for neutron reflectometry studies

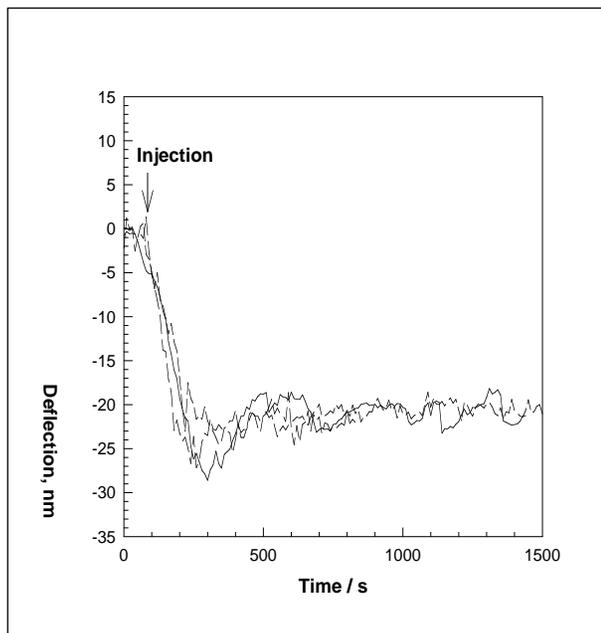
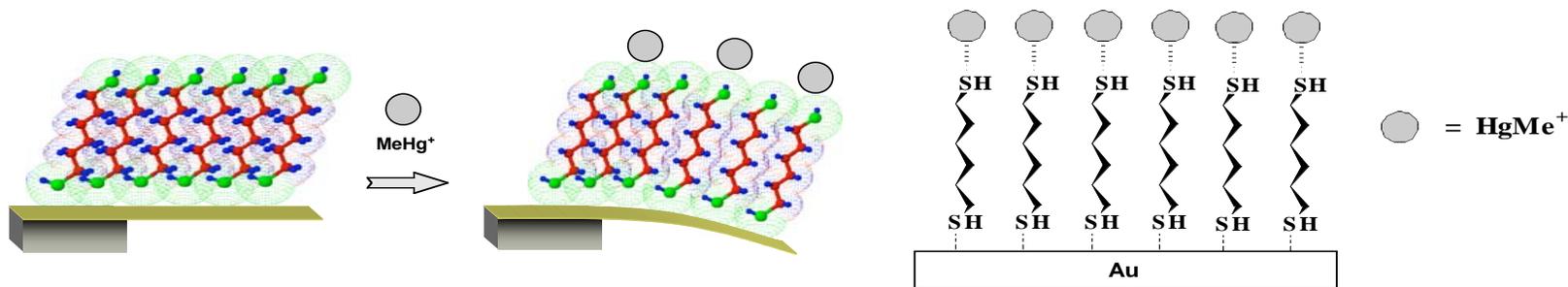


*** Critical understanding of function of cytochromes required for dissimilatory metal reduction**

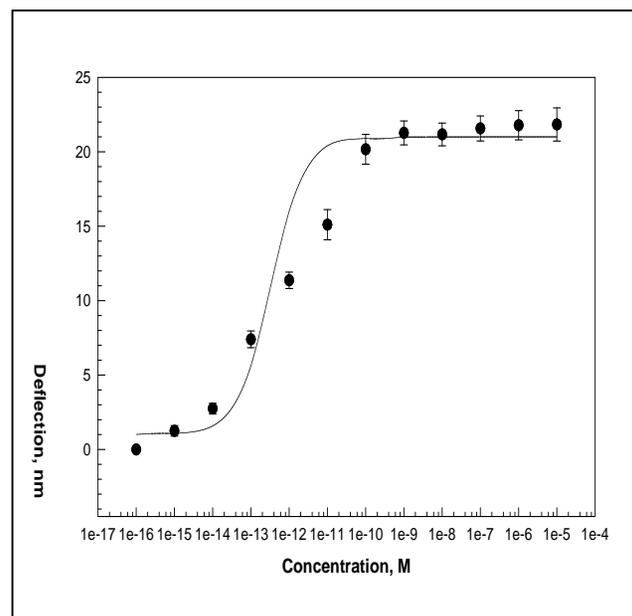
Shi *et al.*, *Journal of Bacteriology*, 188:4705-4714, 2006
 Weber *et al.*, *Nature Reviews Microbiology*, 4(10), 752-764, 2006
 Ross *et al.*, *Applied and Environmental Microbiology*, 73:5797-5808, 2007

Directed research (short term)

High throughput methyl mercury detection using 1,6-Hexanedithiol monolayers modified cantilevers

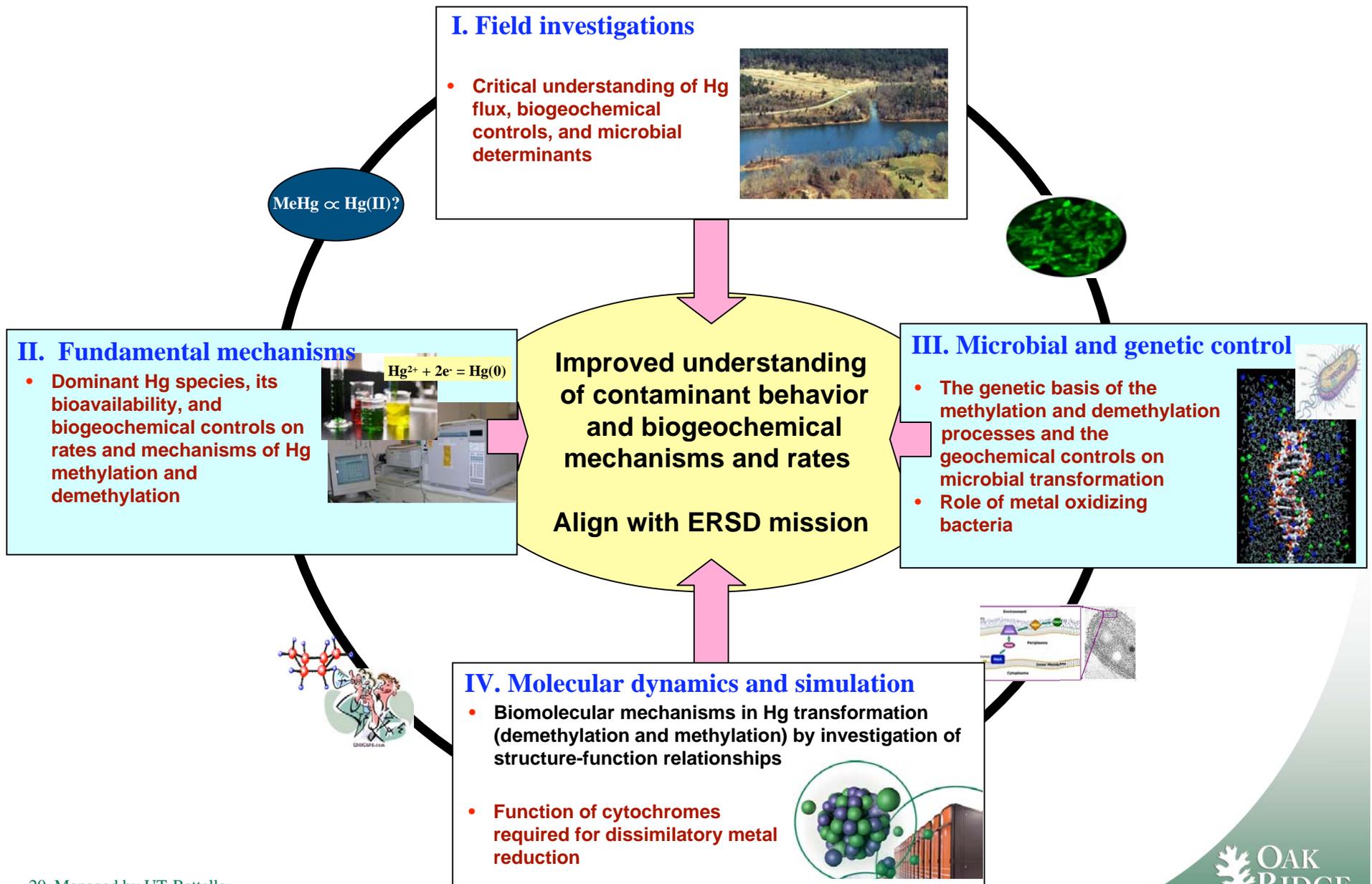


Bending of the cantilever to 1×10^{-6} M of CH_3Hg^+ in water (three experiments)



Bending of this cantilever as a function of the concentration of CH_3Hg^+

Expected deliverables



Partnerships and Collaboration

Key collaborations

- **ORNL task leaders and staff**

Field geochemistry, Brooks, Southworth; Aqueous chemistry, Gu, Miller; Microbiology, Palumbo, Brown, Phelps; Environmental surface chemistry, Liang; Biophysics, Johs; other existing staff as needed, new hires (TBD)

- **University connections-- External Science collaborators**

C. Gilmour (Microbiology, Smithsonian), H. Guo, J Smith (Molecular Dynamics and enzyme simulation, UTK), G. Luther (Sediment sulfide chemistry), S. Miller (Hg molecular biology, UCSF), K. Nagy (coordination chemistry, UIC), L. Shi (protein biochemistry, PNNL), A. Summers (Hg biochemistry, UGA), J. Wall (Microbiology, UMC), H. Zhang (Hg chemistry, TTU)

- **Nat Lab and User Facilities**

T. Droubay (Materials physicist, EMSL), Ken Kemner (EXAFS, APS), K. Littrell (Neutron scattering, ORNL SNS/HFIR), D. Myles (Deuterium labeling, CSMB)

Together with

Advisory panel

Oak Ridge DOE

EM applied science program



Spallation Neutron Source at ORNL

Scientific Impact and DOE Benefits

Understand key Hg precursors for microbial methylation

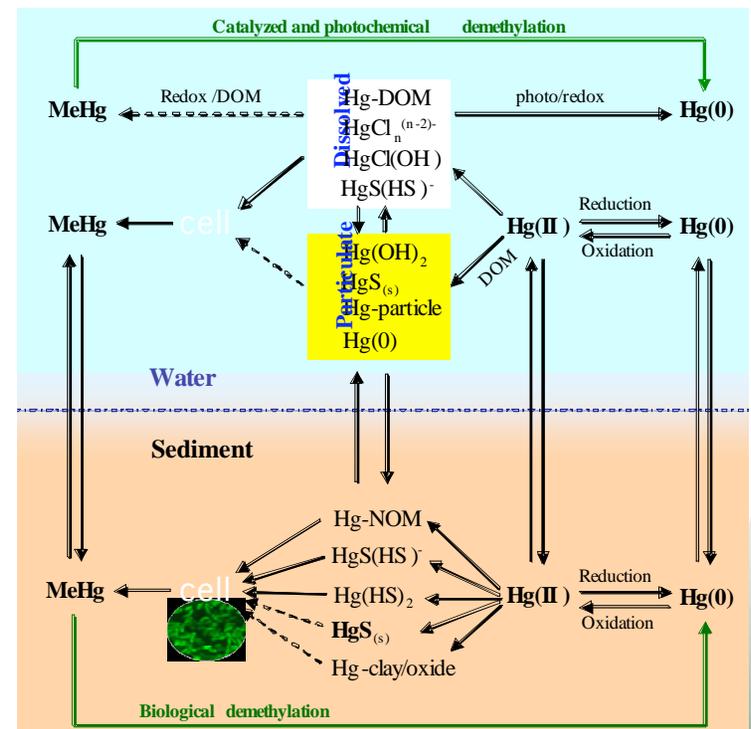
- Geochemical manipulation
- Role of sulfide, thiosulfate, NOM etc influencing Hg speciation
- Catalyzed and photochemical transformation of Hg

Reduce net methylation

- Change biochemistry, microbial processes; ecology
- Stimulate demethylation in microbial community
- Use of genomics sequence data, microarray technology and advanced analytical methods

Other products or contributions

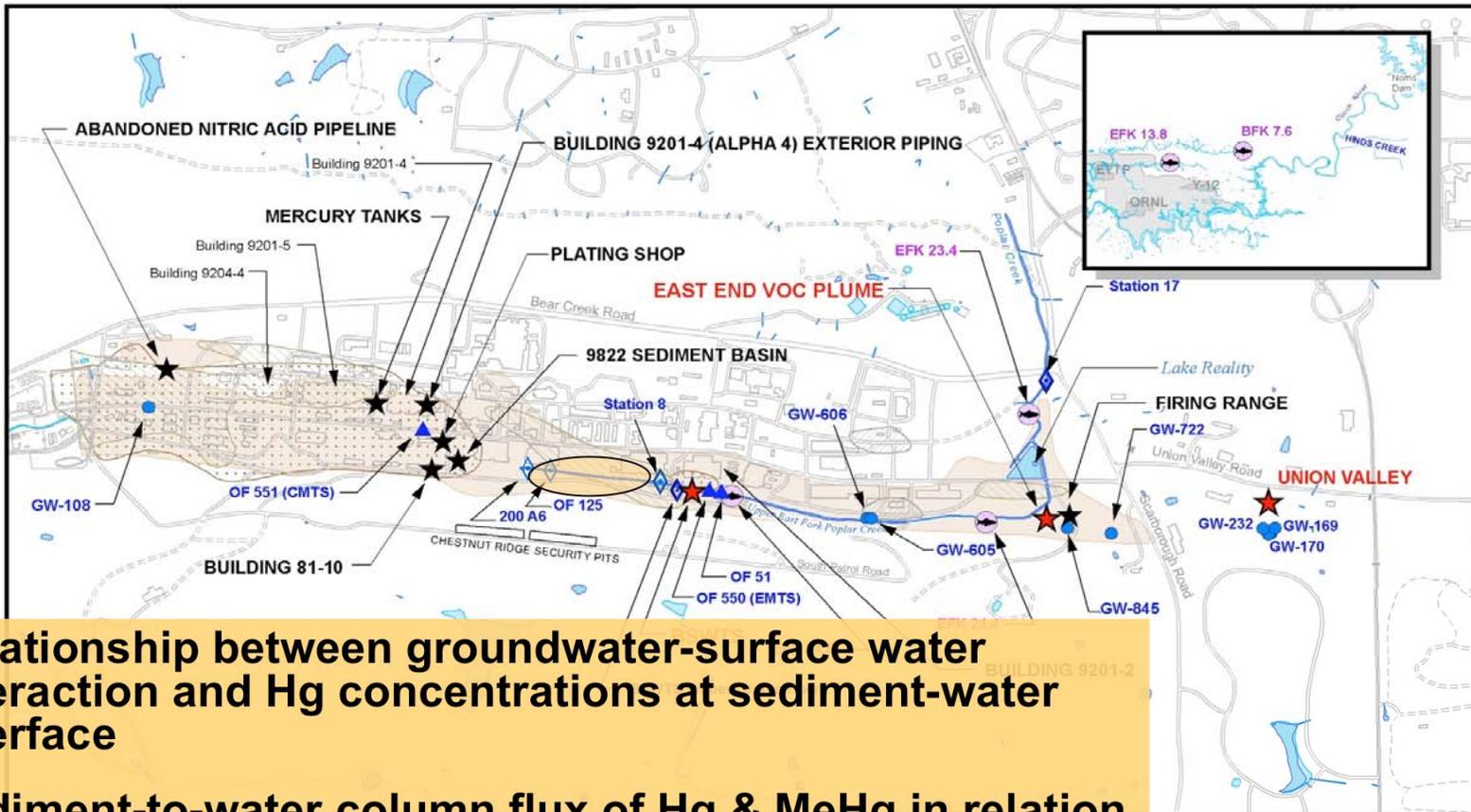
- EM-22 Hg workshop
- Communicate to EM



LDRD and internal investment Supporting the ERSP SFA

- **Tracing Nanoparticle Transport in Porous Media by Neutron Radiography and SANS (LDRD Seed money fund)**
- **ESD –subsurface laboratory renovation**
- **Probing molecular interactions between microbial-cell proteins and mineral surfaces with neutrons (neutron sciences initiative)**
- **(Seed Money Fund)**

Site Investigation



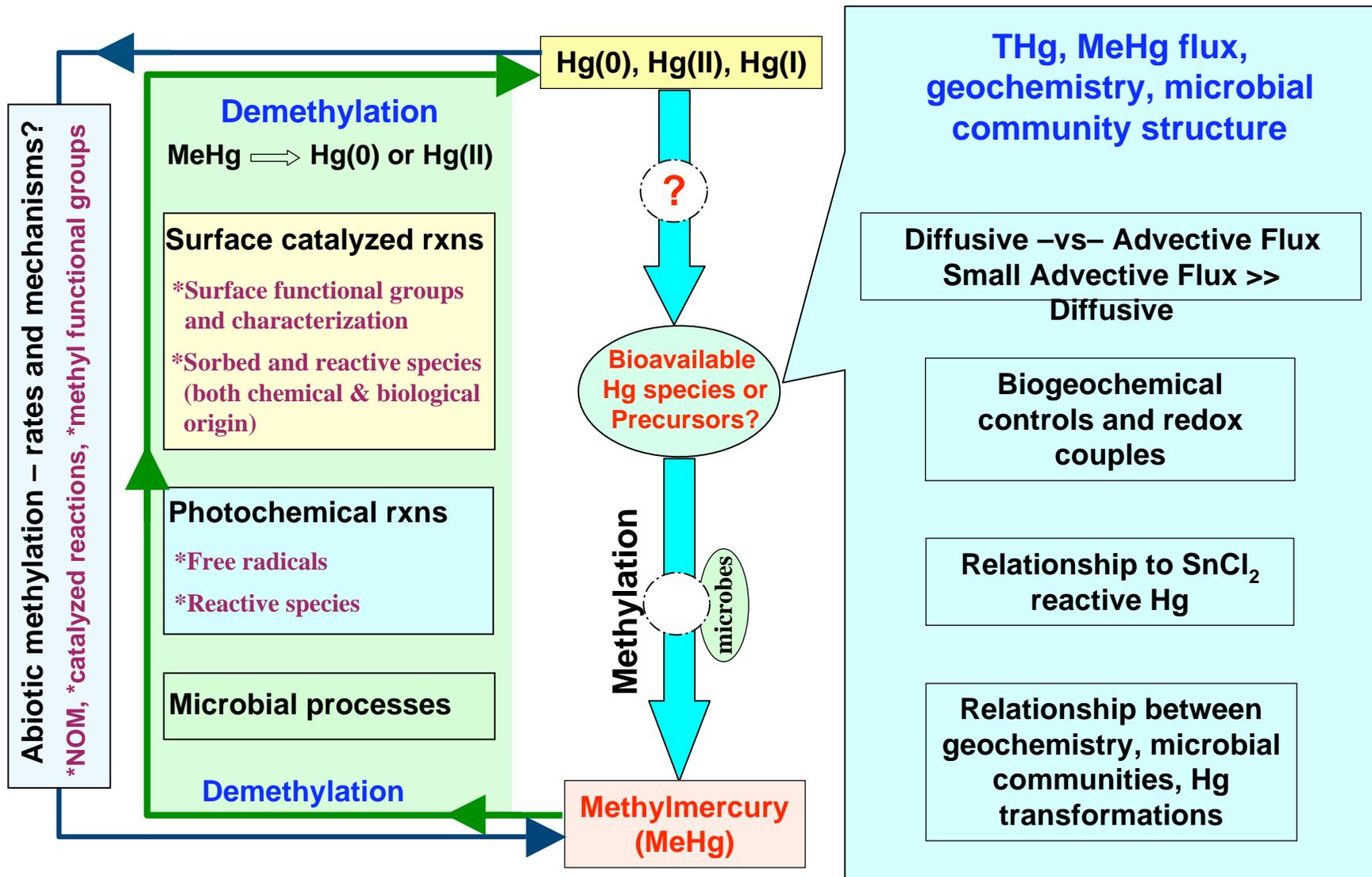
- Relationship between groundwater-surface water interaction and Hg concentrations at sediment-water interface
- Sediment-to-water column flux of Hg & MeHg in relation to water chemistry, biogeochemical gradients, and environmental variables (e.g., photoinduced effects)
- Biogeochemical controls on the transformations that sustain methyl Hg concentrations in water
- Relationships among microbes, community structure, geochemistry, and Hg transformations

OAK RIDGE RESERVATION OAK RIDGE, TENNESSEE

COORDINATE SYSTEM: Oak Ridge Administration Grid
 PROJECTION: Admin.
 DATUM: NAD83 Feet
 DATE: 12/20/07
 MAP DOCUMENT NAME: D52UEFFPC-CERCLA1_08.mxd
 MAP AUTHOR: Carrie Wolfe
 ORGANIZATION: Bechtel Jacobs Company, LLC
 SOURCES: Oak Ridge Environmental Information System
 * "Report on Remedial Investigation of the Upper East Fork Poplar Creek Characterization Area at the Y-12 Plant, Oak Ridge, Tennessee" (DOE/OR/01-1641V3&D2), 1998.

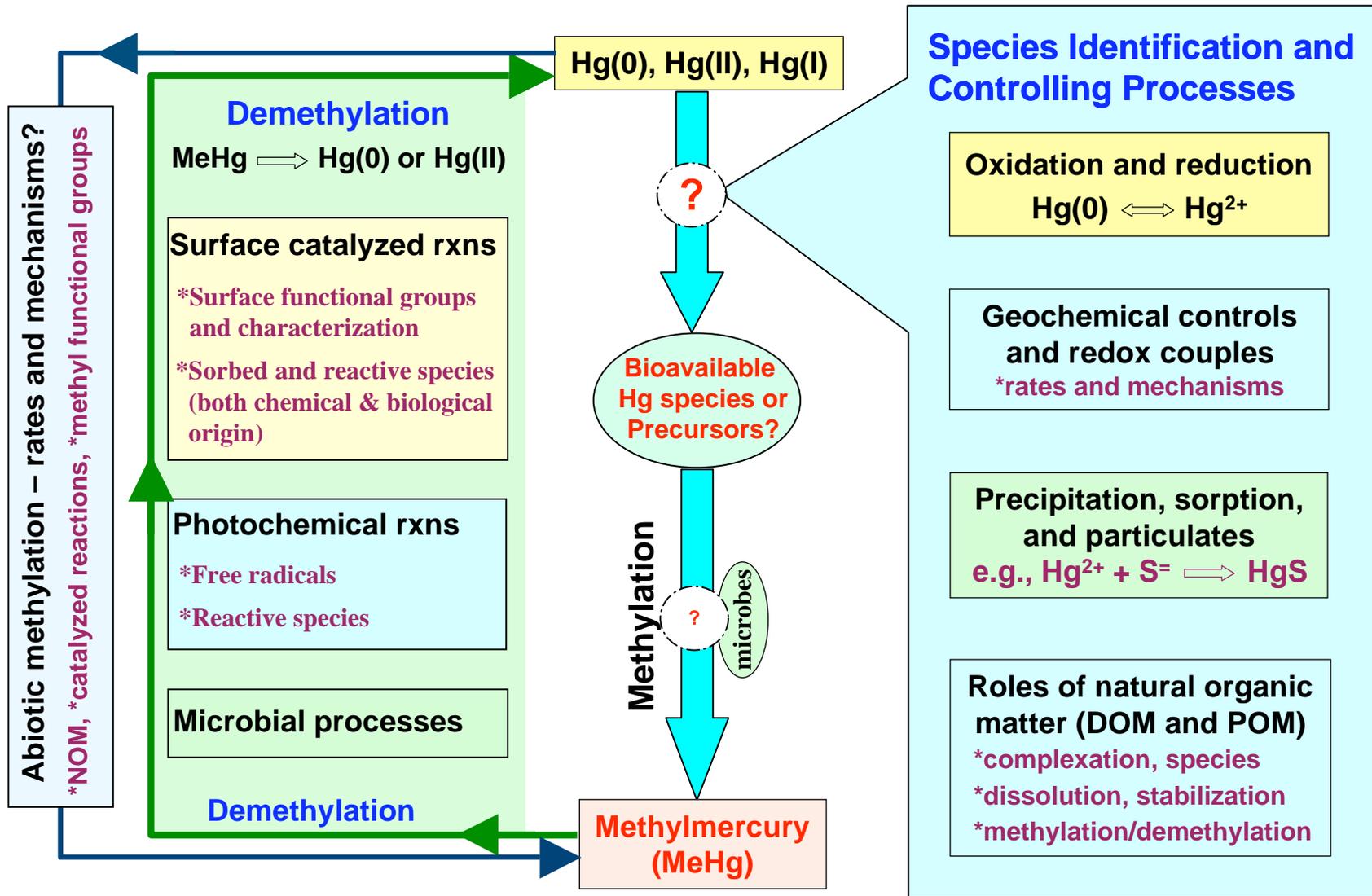
SITE BIOGEOCHEMICAL PROCESSES and MICROCOSM STUDIES

Geochemistry – Microbial Community – Hg Transformations

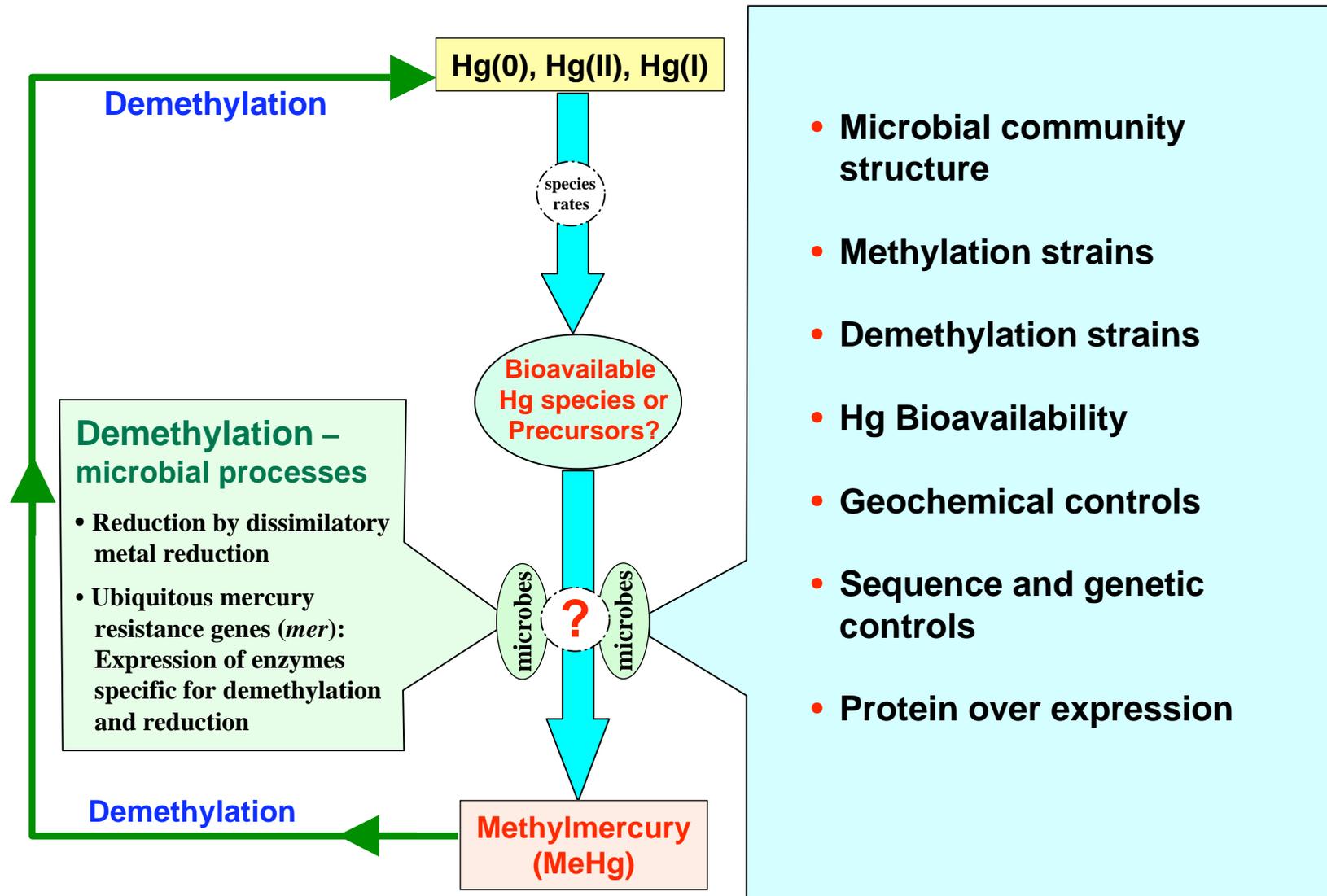


Fundamental mechanisms and transformations

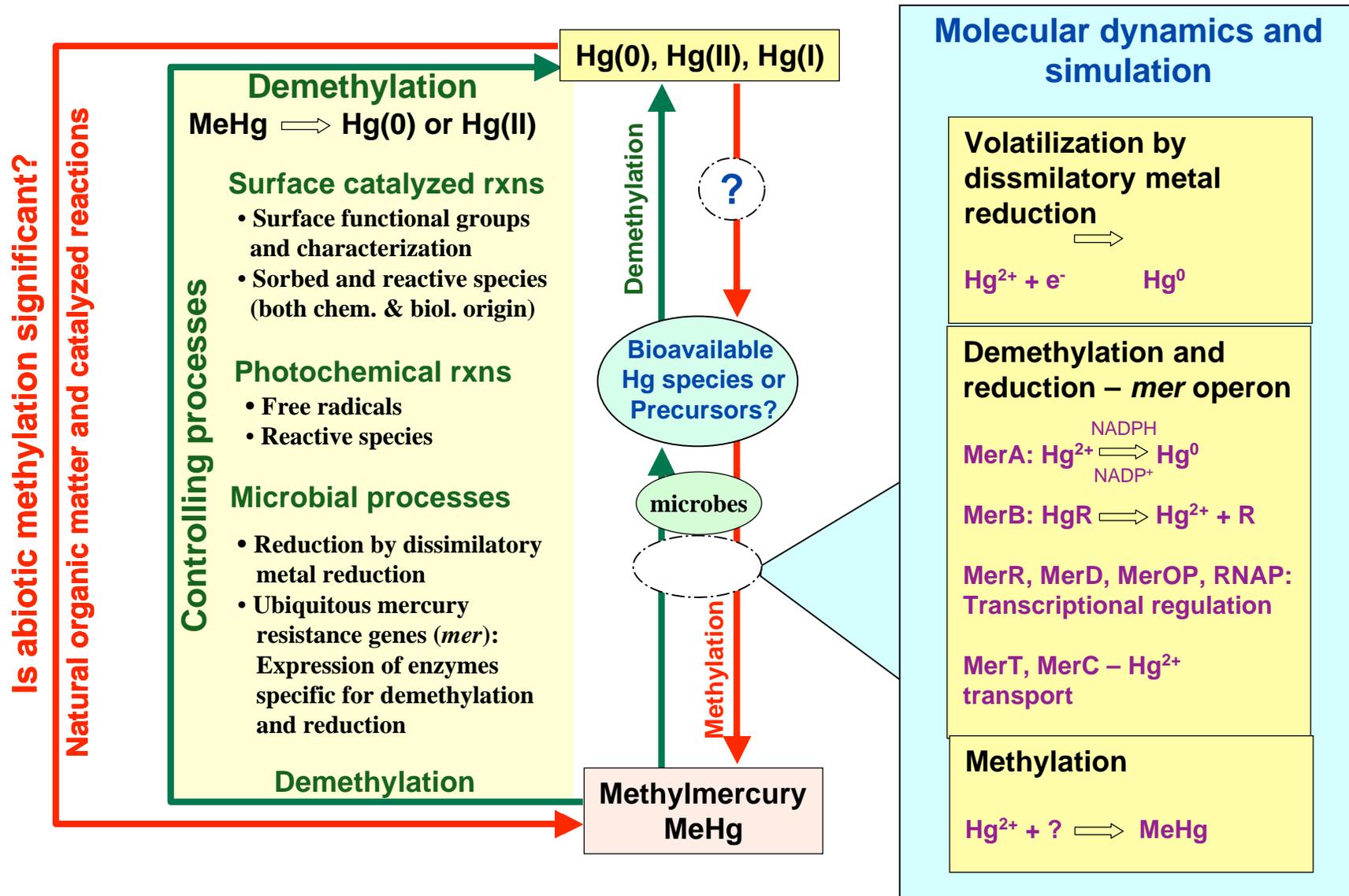
Speciation and Geochemical Controls



Microbial MeHg Production and Genetic Determinants



Integrated tasks



Focus on key Hg biogeochemical processes

Hypotheses

Enzymatic mechanisms of transformation between major Hg species and methyl mercury

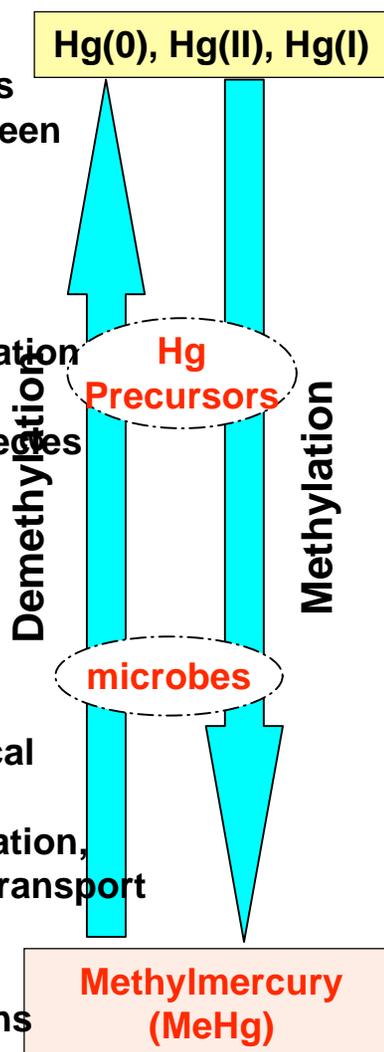
Oxidation, reduction, and species transformation

Dominant chemical species and bioavailability

Biological and abiotic methylation and demethylation

Coupled biogeochemical reactions – sorption, complexation, precipitation, stabilization, fate and transport

Surface catalyzed and photochemical reactions



Task 1: Hg, MeHg flux, geochemistry, microbial community structure

Task 2: Species identification and controlling processes

Task 3: Microbial processes, genes sequence and genetic controls

Task 4: Structure, dynamics, and function of relevant enzymes

Mercury Concentrations in Fish Remain Elevated

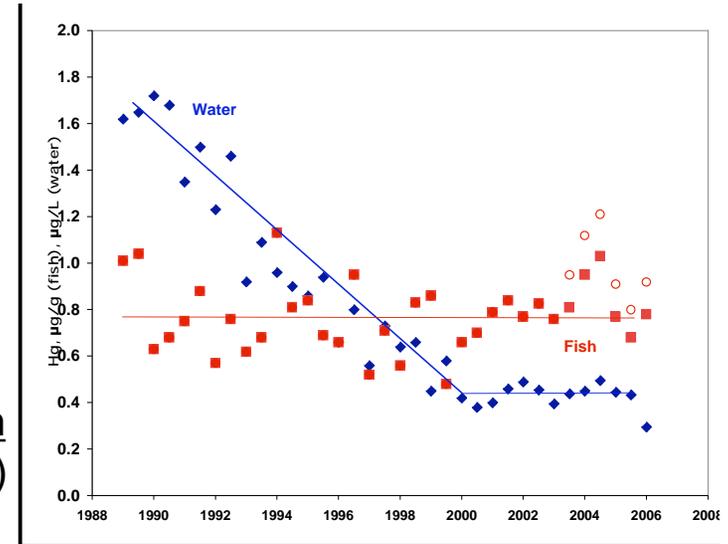
So far, mercury bioaccumulation not proportional to the concentration of waterborne Hg

ORR Examples of water/fish disconnect:

Site	Hg in water (ng/L)	Hg in fish (mg/kg)
White Oak Creek	60	0.5
EF Poplar Cr (upper)	400	0.8
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Reference site	1-3	0.2

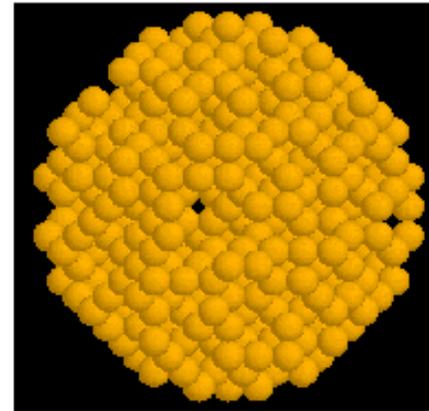
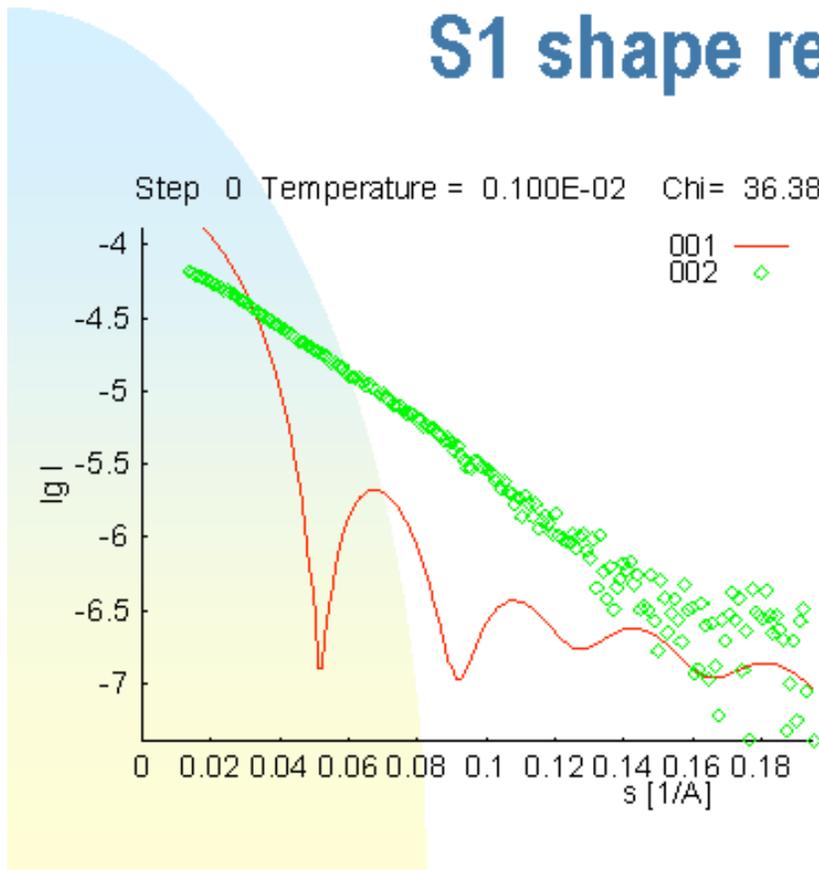
Elimination of inorganic Hg inputs not possible; alternative strategies that reduce methylation in-situ may be the only way to reach fish concentration targets

Basic research needs on mercury methylation at sediment-water interface and particularly what limits methyl mercury production



Low resolution shape reconstruction from SANS/SAXS data

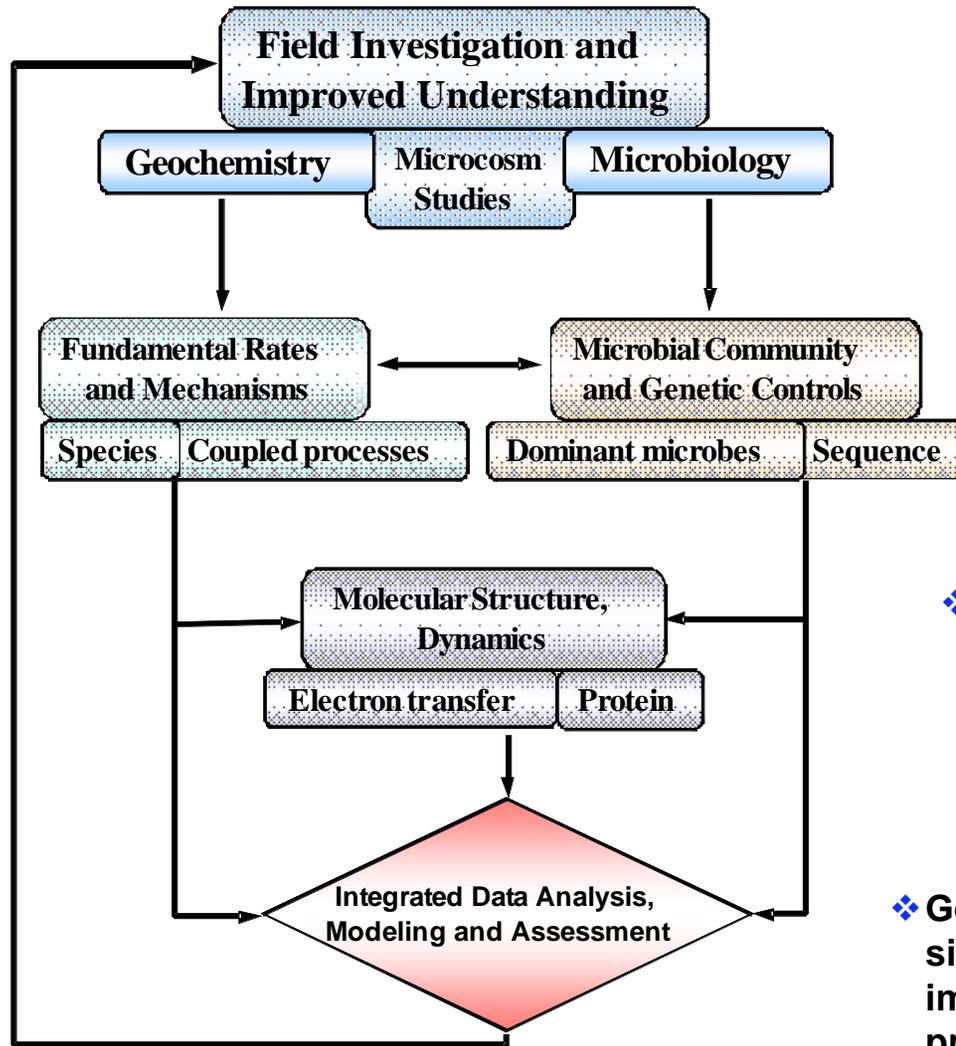
S1 shape reconstruction



$$\chi^2 = \frac{1}{N_p - 1} \sum_i \left[\frac{I(q_i)_{\text{exp.}} - cI(q_i)_{\text{calc.}}}{\sigma(q_i)} \right]^2$$

- *Ab initio* shape reconstruction by DAMMIN.
(D. Svergun, Biophys J. 76: 2879-2886, 1999)

Data flow and integration

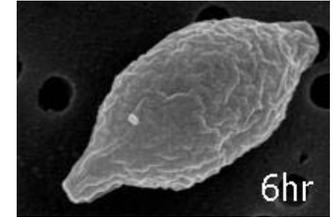


- ❖ Determination of site geochemistry, dominant chemical species and microbial community provides critical information for controlled laboratory mechanistic studies
 - ↓
- ❖ Fundamental understanding of Hg species transformation, geochemical controls, and genetic determinants essential for methylation and demethylation processes
 - ↓
- ❖ Critical understanding of biomolecular mechanisms in Hg transformation (demethylation and methylation) using structure-functional relationships and genetic regulation
 - ↓
- ❖ Geochemical modeling, molecular simulation, and data integration for improved understanding of field processes and remedial controls

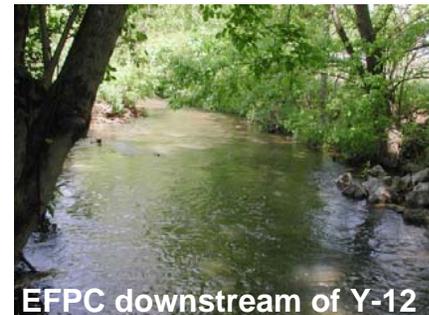
Microbial MeHg Production

Scientific Issues Addressed

- **Elucidate the genetic determinants of MeHg production and regulation.**
 - **Comparative gene expression, mutagenesis, and complementation.**
- **Determine the effect of geochemical factors on gene regulatory networks for mercury methylation.**
 - **Use whole genome microarrays to examine both biotic and abiotic effects on the methylating and nonmethylating *Desulfovibrio* transcriptomic profiles.**
- **Examine relationships among community structure, geochemical conditions, and MeHg production in sediments collected from Hg-contaminated sites.**
 - **Functional gene arrays**
 - **16S rRNA gene clone library analysis**



Desulfovibrio africanus
(SEM by Dwayne Elias, U of Missouri)



EFPC downstream of Y-12